'AL USE ONLY

ACCESS DB # 158779

PLEASE PRINT CLEARLY

Scientific and Technical Information Center

SEARCH REQUEST FORM

ll Name: JANE ZARA Examiner # : 7751 Date: 7/11/05

.35 Phone Number: 2-0765 Serial Number: 09/788,076

Room#): 2D28 (Mailbox #): 2C18 Results Format Preferred (circle): PAPER DISK

****

ent and quality search, please attach a copy of the cover sheet, claims, and abstract or fill out the following:

on: Method of Drug Analysis

e provide full names): HOTA MISL, GIC, GS

y Date: 2/16/01

tailed statement of the search topic, and describe as specifically as possible the subject matter to be searched. Include the tructures, keywords, synonyms, acronyms, and registry numbers, and combine with the concept or utility of the invention. 'hat may have a special meaning. Give examples or relevant citations, authors, etc., if known.

arches Only* *Please include all pertinent information (parent, child, divisional, or issued patent numbers) along with the number.*

CRFC

Please Search Seq ID Nos

2 + 4

— Full No size restrictions

— Please include interfering strands.

Thanks.

****

E ONLY

D. Schreiber

272-2526

: Remsen E01/4C1

ked Up:

7/14

Review Time: 14

7

Type of Search

2 NA Sequence (#)

AA Sequence (#)

Structure (#)

Bibliographic

Litigation

Fulltext

Other

Vendors and cost where applicable

STN Dialog

Questel/Orbit Lexis/Nexis

Westlaw WWW/Internet

✓ In-house sequence systems Compugen

✓ Commercial Oligomer Score/Length

✓ Interference SPDI Encode/Transl

Other (specify)

ACCESS DB # _____

FOR OFFICIAL USE ONLY *PLEASE PRINT CLEARLY*

Scientific and Technical Information Center

SEARCH REQUEST FORM

Requester's Full Name: JANE ZARA Examiner #: 7751 Date: 7/11/05
Art Unit: 1635 Phone Number: 2-0765 Serial Number: 09/788,074
Location (Bldg/Room#): 2D28 (Mailbox #): 2C19 Results Format Preferred (circle): PAPER DISK

****

To ensure an efficient and quality search, please attach a copy of the cover sheet, claims, and abstract or fill out the following:

Title of Invention: Method of Drug Analysis

Inventors (please provide full names): HOTA MISRA, GIC, GS

Earliest Priority Date: 2/16/01

Search Topic:
Please provide a detailed statement of the search topic, and describe as specifically as possible the subject matter to be searched. Include the elected species or structures, keywords, synonyms, acronyms, and registry numbers, and combine with the concept or utility of the invention. Define any terms that may have a special meaning. Give examples or relevant citations, authors, etc., if known.

For Sequence Searches Only ***Please include all pertinent information (parent, child, divisional, or issued patent numbers) along with the appropriate serial number.***

Please Search Seq ID Nos

2 + 4

— No size restrictions

— Please include interferences.

Thanks.

****

STAFF USE ONLY

Searcher: _____
Searcher Phone #: _____
Searcher Location: _____
Date Searcher Picked Up: _____
Date Completed: _____
Searcher Prep & Review Time: _____
Online Time: _____

Type of Search

_____ NA Sequence (#)
_____ AA Sequence (#)
_____ Structure (#)
_____ Bibliographic
_____ Litigation
_____ Fulltext
_____ Other

Vendors and cost where applicable

_____ STN _____ Dialog
_____ Questel/Orbit _____ Lexis/Nexis
_____ Westlaw _____ WWW/Internet
____ In-house sequence systems
__ Commercial ___ Oligomer ____ Score/Length
__ Interference ___ SPDI ____ Encode/Transl
_____ Other (specify)

File 5:Biosis Previews(R) 1969-2005/Jul W2
(c) 2005 BIOSIS
File 6:NTIS 1964-2005/Jul W2
(c) 2005 NTIS, Intl Cpyrght All Rights Res
File 8:Ei Compendex(R) 1970-2005/Jul W2
(c) 2005 Elsevier Eng. Info. Inc.
File 24:CSA Life Sciences Abstracts 1966-2005/Jun
(c) 2005 CSA.
File 34:SciSearch(R) Cited Ref Sci 1990-2005/Jul W2
(c) 2005 Inst for Sci Info
File 65:Inside Conferences 1993-2005/Jul W2
(c) 2005 BLDSC all rts. reserv.
File 71:ELSEVIER BIOBASE 1994-2005/Jul W2
(c) 2005 Elsevier Science B.V.
File 73:EMBASE 1974-2005/Jul 15
(c) 2005 Elsevier Science B.V.
File 94:JICST-EPlus 1985-2005/May W5
(c)2005 Japan Science and Tech Corp(JST)
File 98:General Sci Abs/Full-Text 1984-2004/Dec
(c) 2005 The HW Wilson Co.
File 99:Wilson Appl. Sci & Tech Abs 1983-2005/Jun
(c) 2005 The HW Wilson Co.
File 135:NewsRx Weekly Reports 1995-2005/Jul W2
(c) 2005 NewsRx
File 143:Biol. & Agric. Index 1983-2005/Jun
(c) 2005 The HW Wilson Co
File 144:Pascal 1973-2005/Jul W1
(c) 2005 INIST/CNRS
File 155:MEDLINE(R) 1951-2005/Jul W3
(c) format only 2005 The Dialog Corp.
File 172:EMBASE Alert 2005/Jul 15
(c) 2005 Elsevier Science B.V.
File 266:FEDRIP 2005/Jun
Comp & dist by NTIS, Intl Copyright All Rights Res
File 315:ChemEng & Biotec Abs 1970-2005/Jun
(c) 2005 DECHEMA
File 357:Derwent Biotech Res. _1982-2005/Jul W3
(c) 2005 Thomson Derwent & ISI
File 358:Current BioTech Abs 1983-2005/Jun
(c) 2005 DECHEMA
File 369:New Scientist 1994-2005/May W3
(c) 2005 Reed Business Information Ltd.
File 370:Science 1996-1999/Jul W3
(c) 1999 AAAS
File 399:CA SEARCH(R) 1967-2005/UD=14304
(c) 2005 American Chemical Society
File 434:SciSearch(R) Cited Ref Sci 1974-1989/Dec
(c) 1998 Inst for Sci Info
File 40:Enviroline(R) 1975-2005/Jun
File 50:CAB Abstracts 1972-2005/Jun
(c) 2005 CAB International
File 103:Energy SciTec 1974-2005/Jun B2
(c) 2005 Contains copyrighted material
File 156:ToxFile 1965-2005/Jul W3
(c) format only 2005 The Dialog Corporation
File 162:Global Health 1983-2005/Jun
(c) 2005 CAB International
File 305:Analytical Abstracts 1980-2005/Jul W1
(c) 2005 Royal Soc Chemistry
File 393:Beilstein Abstracts 2005/Q2
(c) Beilstein GmbH
File 35:Dissertation Abs Online 1861-2005/Jun
(c) 2005 ProQuest Info&Learning

```
File  48:SPORTDiscus 1962-2005/Jan
         (c) 2005 Sport Information Resource Centre
File  91:MANTIS(TM) 1880-2005/Jun
         2001 (c) Action Potential
File 149:TGG Health&Wellness DB(SM) 1976-2005/Jul W2
         (c) 2005 The Gale Group
File 159:Cancerlit 1975-2002/Oct
         (c) format only 2002 Dialog Corporation
File 164:Allied & Complementary Medicine 1984-2005/Jul
          (c) 2005 BLHCIS
File 444:New England Journal of Med. 1985-2005/Jul W1
         (c) 2005 Mass. Med. Soc.
File 467:ExtraMED(tm) 2000/Dec
         (c) 2001 Informania Ltd.

Set     Items   Description
S1        668   AU=( HOTAMISLIGIL G? OR HOTAMISLIGIL, G?)
S2        448   S1 AND (DIABET? OR INSULIN)
S3          5   S2 AND MAL
S4          3   RD (unique items)
```

```
? show files;ds;t/3,k/all
File   5:Biosis Previews(R) 1969-2005/Jul W2
         (c) 2005 BIOSIS
File   6:NTIS 1964-2005/Jul W2
         (c) 2005 NTIS, Intl Cpyrght All Rights Res
File   8:Ei Compendex(R) 1970-2005/Jul W2
         (c) 2005 Elsevier Eng.  Info. Inc.
File  24:CSA Life Sciences Abstracts 1966-2005/Jun
         (c) 2005 CSA.
File  34:SciSearch(R) Cited Ref Sci 1990-2005/Jul W2
         (c) 2005 Inst for Sci Info
File  65:Inside Conferences 1993-2005/Jul W2
         (c) 2005 BLDSC all rts. reserv.
File  71:ELSEVIER BIOBASE 1994-2005/Jul W2
         (c) 2005  Elsevier Science B.V.
File  73:EMBASE 1974-2005/Jul 15
         (c) 2005 Elsevier Science B.V.
File  94:JICST-EPlus 1985-2005/May W5
         (c)2005 Japan Science and Tech Corp(JST)
File  98:General Sci Abs/Full-Text 1984-2004/Dec
         (c) 2005 The HW Wilson Co.
File  99:Wilson Appl. Sci & Tech Abs 1983-2005/Jun
         (c) 2005 The HW Wilson Co.
File 135:NewsRx Weekly Reports 1995-2005/Jul W2
         (c) 2005 NewsRx
File 143:Biol. & Agric. Index 1983-2005/Jun
         (c) 2005 The HW Wilson Co
File 144:Pascal 1973-2005/Jul W1
         (c) 2005 INIST/CNRS
File 155:MEDLINE(R) 1951-2005/Jul W3
         (c) format only 2005 The Dialog Corp.
File 172:EMBASE Alert 2005/Jul 15
         (c) 2005 Elsevier Science B.V.
File 266:FEDRIP 2005/Jun
         Comp & dist by NTIS, Intl Copyright All Rights Res
File 315:ChemEng & Biotec Abs 1970-2005/Jun
         (c) 2005 DECHEMA
File 357:Derwent Biotech Res. _1982-2005/Jul W3
         (c) 2005 Thomson Derwent & ISI
File 358:Current BioTech Abs 1983-2005/Jun
          (c) 2005 DECHEMA
File 369:New Scientist 1994-2005/May W3
         (c) 2005 Reed Business Information Ltd.
File 370:Science 1996-1999/Jul W3
         (c) 1999 AAAS
File 399:CA SEARCH(R) 1967-2005/UD=14304
         (c) 2005 American Chemical Society
File 434:SciSearch(R) Cited Ref Sci 1974-1989/Dec
         (c) 1998 Inst for Sci Info
File  40:Enviroline(R) 1975-2005/Jun
File  50:CAB Abstracts 1972-2005/Jun
         (c) 2005 CAB International
File 103:Energy SciTec 1974-2005/Jun B2
         (c) 2005 Contains copyrighted material
File 156:ToxFile 1965-2005/Jul W3
         (c) format only 2005 The Dialog Corporation
File 162:Global Health 1983-2005/Jun
         (c) 2005 CAB International
File 305:Analytical Abstracts 1980-2005/Jul W1
         (c) 2005 Royal Soc Chemistry
File 393:Beilstein Abstracts 2005/Q2
         (c) Beilstein GmbH
```

File 35:Dissertation Abs Online 1861-2005/Jun
        (c) 2005 ProQuest Info&Learning
File 48:SPORTDiscus 1962-2005/Jan
        (c) 2005 Sport Information Resource Centre
File 91:MANTIS(TM) 1880-2005/Jun
        2001 (c) Action Potential
File 149:TGG Health&Wellness DB(SM) 1976-2005/Jul W2
        (c) 2005 The Gale Group
File 159:Cancerlit 1975-2002/Oct
        (c) format only 2002 Dialog Corporation
File 164:Allied & Complementary Medicine 1984-2005/Jul
         (c) 2005 BLHCIS
File 444:New England Journal of Med. 1985-2005/Jul W1
        (c) 2005 Mass. Med. Soc.
File 467:ExtraMED(tm) 2000/Dec
        (c) 2001 Informania Ltd.

Set	Items	Description
S1	668	AU=(HOTAMISLIGIL G? OR HOTAMISLIGIL, G?)
S2	448	S1 AND (DIABET? OR INSULIN)
S3	5	S2 AND MAL
S4	3	RD (unique items)

>>>KWIC option is not available in file(s): 399

 4/3,K/1   (Item 1 from file: 5)
DIALOG(R)File   5:Biosis Previews(R)
(c) 2005 BIOSIS. All rts. reserv.

0014189746   BIOSIS NO.: 200300148465
Role of the fatty acid binding protein mal1 in obesity and **insulin** resistance.
AUTHOR: Maeda Kazuhisa; Uysal K Teoman; Makowski Liza; Gorgun Cem Z; Atsumi Genichi; Parker Rex A; Bruning Jens; Hertzel Ann Vogel; Bernlohr David A; **Hotamisligil Gokhan S** (Reprint
AUTHOR ADDRESS: Harvard School of Public Health, 665 Huntington Ave., Boston, MA, 02115, USA**USA
AUTHOR E-MAIL ADDRESS: ghotamis@hsph.harvard.edu
JOURNAL: Diabetes 52 (2): p300-307 February 2003 2003
MEDIUM: print
ISSN: 0012-1797 _(ISSN print)
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

Role of the fatty acid binding protein mal1 in obesity and **insulin** resistance.
...AUTHOR: ***Hotamisligil Gokhan S***

ABSTRACT: The metabolic syndrome is a cluster of metabolic and inflammatory abnormalities including obesity, **insulin** resistance, type 2 ***diabetes*** , hypertension, dyslipidemia, and atherosclerosis. The fatty acid binding proteins aP2 (fatty acid binding protein (FABP)-4) and mal1 (FABP5) are closely related and both are expressed in adipocytes. Previous studies in aP2-deficient mice have indicated a significant role for aP2 in obesity-related **insulin** resistance, type 2 ***diabetes*** , and atherosclerosis. However, the biological functions of mal1 are not known. Here, we report the generation of mice with targeted null mutations in the mal1...

...address the role of the second adipocyte FABP in metabolic regulation in the presence and deficiency of obesity, absence of mal1 resulted in increased systemic **insulin** sensitivity in two models of obesity and ***insulin*** resistance. Adipocytes isolated from mal1-deficient mice

also exhibited enhanced **insulin**-stimulated glucose transport capacity. In contrast, mice expressing high levels of mall in adipose tissue display reduced systemic ***insulin*** sensitivity. Hence, our results demonstrate that mall modulates adipose tissue function and contributes to systemic glucose metabolism and constitutes a potential therapeutic target in ***insulin*** resistance.
...REGISTRY NUMBERS: ***insulin***
DESCRIPTORS:
CHEMICALS & BIOCHEMICALS: ***insulin*** --...

... ***mal*** -1 fatty acid binding protein...

... ***insulin*** resistance development role, obesity development role, adipose tissue function modulatory role, systemic glucose metabolism role

4/3,K/2 (Item 1 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

135200396 CA: 135(14)200396b PATENT
Treatment lipid metabolic disorders by inhibiting Mall, the keratinocyte lipid binding protein activity
INVENTOR(AUTHOR): Hotamisligil, Gokhan S.
LOCATION: USA
ASSIGNEE: President and Fellows of Harvard College
PATENT: PCT International ; WO 200160384 A1 DATE: 20010823
APPLICATION: WO 2001US5019 (20010216) *US PV183106 (20000217)
PAGES: 27 pp. CODEN: PIXXD2 LANGUAGE: English CLASS: A61K-031/7088A; A61K-039/395B; A61P-003/06B; A61P-003/10B; A61P-009/10B; G01N-033/50B
DESIGNATED COUNTRIES: AE; AG; AL; AM; AT; AU; AZ; BA; BB; BG; BR; BY; BZ; CA; CH; CN; CR; CU; CZ; DE; DK; DM; DZ; EE; ES; FI; GB; GD; GE; GH; GM; HR; HU; ID; IL; IN; IS; JP; KE; KG; KP; KR; KZ; LC; LK; LR; LS; LT; LU; LV; MA; MD; MG; MK; MN; MW; MX; MZ; NO; NZ; PL; PT; RO; RU; SD; SE; SG; SI; SK; SL; TJ; TM; TR; TT; TZ; UA; UG; US; UZ; VN; YU; ZA; ZW; AM; AZ; BY; KG; KZ; MD; RU; TJ; TM DESIGNATED REGIONAL: GH; GM; KE; LS; MW; MZ; SD; SL; SZ; TZ; UG ; ZW; AT; BE; CH; CY; DE; DK; ES; FI; FR; GB; GR; IE; IT; LU; MC; NL; PT; SE; TR; BF; BJ; CF; CG; CI; CM; GA; GN; GW; ML; MR; NE; SN; TD; TG

4/3,K/3 (Item 1 from file: 149)
DIALOG(R)File 149:TGG Health&Wellness DB(SM)

01863324 SUPPLIER NUMBER: 56456896 (USE FORMAT 7 OR 9 FOR FULL TEXT)
Altered **Insulin** Secretion Associated With Reduced Lipolytic Efficiency in (aP2.sup.-/-) Mice.
Scheja, Ludger; Makowski, Liza; Uysal, K. Teoman; Wiesbrock, Sarah M.; Shimshek, Derya R.; Meyers, Daniel S.; Morgan, Maureen; Parker, Rex A.; ***Hotamisligil, Gokhan S.***
Diabetes, 48, 10, 1987
Oct,
1999
PUBLICATION FORMAT: Magazine/Journal; Refereed ISSN: 0012-1797
LANGUAGE: English RECORD TYPE: Fulltext TARGET AUDIENCE: Professional
WORD COUNT: 8092 LINE COUNT: 00671

Altered **Insulin** Secretion Associated With Reduced Lipolytic Efficiency in (aP2.sup.-/-) Mice.
... ***Hotamisligil, Gokhan S.***

TEXT:

...fatty acid-binding protein (aP2) results in minor alterations of plasma lipids and adipocyte development but provides significant protection from dietary obesity-induced hyperinsulinemia and ***insulin*** resistance. To identify potential mechanisms responsible for this phenotype, we examined lipolysis and ***insulin*** secretion in (aP2.sup.-/-) mice. (Beta)-Adrenergic stimulation resulted in a blunted rise of blood glycerol levels in (aP2.sup.-/-) compared with (aP2.sup.+/+) mice...

...The decreased lipolytic response seen in the (aP2.sup.-/-) mice was not associated with altered expression levels of hormone-sensitive lipase or perilipin. The acute ***insulin*** secretory response to (Beta)-adrenergic stimulation was also profoundly suppressed in (aP2.sup.-/-) mice despite comparable total concentrations and only minor changes in the composition ...

...reduction in both stearic and cis-11-eicoseneic acids and an increase in palmitoleic acid were observed. The response of (aP2.sup.-/-) mice to other **insulin** secretagogues such as arginine and glyburide was similar to that of (aP2.sup.+/+) mice, arguing against generally impaired function of pancreatic (Beta)-cells. Finally, no...

...an adipo-pancreatic axis, the proper action of which relies on the presence of aP2. Consequently, aP2's role in the pathogenesis of type 2 **diabetes** might involve regulation of both hyperinsulinemia and **insulin** resistance through its impact on both lipolysis and ***insulin*** secretion. ***Diabetes*** 48:1987-1994, 1999
... aP2.sup.-/-) animals were placed on a high-fat diet to induce obesity (14). The obese (aP2.sup.-/-) mice were significantly protected from hyperinsulinemia and **insulin** resistance compared with their wild-type ((aP2.sup.+/+)) littermates. This protection from dietary obesity-induced **insulin** resistance in the absence of aP2 suggests that adipocyte fatty acid metabolism is a critical component of the mechanisms leading to systemic ***insulin*** resistance in obesity.

Among potentially relevant parameters of adipocyte fatty acid metabolism, the lipolytic breakdown of stored triglycerides is of particular interest. Major physiologic regulation...

...release from adipose tissue are commonly increased in obesity. This increase has been suggested to be an important factor in the initiation of hyperinsulinemia and ***insulin*** resistance (23). In support of this hypothesis, experimental alterations in FFA metabolism have been demonstrated to influence both **insulin** secretion and peripheral ***insulin*** action (24-27). The release of fatty acids from adipocytes could involve FABP activity at several stages, including the efficiency of flux from the lipid droplet to the plasma membrane or intermediary metabolic activities. Hence, we examined the effects of aP2 deficiency on adipocyte lipolysis and the subsequent acute **insulin** secretory response. Our results demonstrate that aP2 plays an important role in regulation of both these parameters.

RESEARCH DESIGN AND METHODS

Animals and experimental conditions...

...in aliquots and stored at -20 (degrees) C. Enzymatic assays were used for the determination of serum FFAs (Wako, Richmond, VA) and glycerol (Sigma). Serum ***insulin*** , C-peptide, and leptin levels were measured using radioimmunoassays from Linco Research (St. Charles, MO). Glucose levels were determined using blood glucose strips (MediSense, Waltham... analysis as described (33). The cDNA clones used for the generation of labeled probes (HSL, perilipin) were provided by Dr. C. Londos, National Institute of **Diabetes** and Digestive and Kidney Diseases, National Institutes of Health. A human ribosomal protein probe (36B4) (34) was used as loading control.

Reverse transcription-polymerase chain...

...mice. Lipolysis is an adipocyte function likely to be altered in (aP2.sup.-/-) mice and also a factor that might contribute to the protection from ***insulin*** resistance observed in these animals. To test this hypothesis in (aP2.sup.-/-) and (aP2.sup.+/+) mice, we first examined lipolysis in vivo using intraperitoneal administration...
...tissue (40-42). In a subset of experiments, we also used isoproterenol (10 mg/kg), a general (Beta)-adrenergic agonist. Circulating levels of FFAs and **insulin** have been shown to be maximally elevated between 10 and 15 min after intraperitoneal administration of CL 316,243 (43) (see below). In line with...

...basal was lower in the (aP2.sup.-/-) mice.
(Figure 1 ILLUSTRATION OMITTED)
In the postprandial state, lipolysis is suppressed because of the negative action of ***insulin*** (44,45). We next tested whether an attenuation of lipolysis could also be detected in the (aP2.sup.-/-) mice under this physiologic condition by performing...

...As expected, basal FFA and glycerol levels in the postprandial state were decreased compared with resting animals, consistent with the observed elevated basal levels of ***insulin*** . The basal levels of both glycerol and FFAs were similar in (aP2.sup.-/-) and (aP2.sup.+/+) animals (Table 1). After administration of CL 316,243...

mmol/l)	0.31 (+ or -) 0.06	0.98 (+ or -) 0.09
Glycerol (mmol/l)	0.18 (+ or -) 0.01	0.42 (+ or -) 0.02
Insulin	(ng/ml) 3.2 (+ or -) 0.96	8.4 (+ or -) 2.4
Glucose (mg/dl)	174 (+ or -) 6.0	148 (+ or -) 9.8
Leptin (ng...		

...mmol/l)	0.26 (+ or -) 0.04	0.80 (+ or -) 0.08
Glycerol (mmol/l)	0.18 (+ or -) 0.03	0.35 (+ or -) 0.02(*)
Insulin	(ng/ml) 2.4 (+ or -) 0.39	3.3 (+ or -) 0.9(*)
Glucose (mg/dl)	161 (+ or -) 8.2	169 (+ or -) 15.1
Leptin (ng...		

...gel electrophoresis was used to determine expression of aP2 and mall in (aP2.sup.-/-) and (aP2.sup.+/+) adipocytes (data not shown).
(Figure 3 ILLUSTRATION OMITTED)
Insulin secretion. (Beta)-Adrenergic stimulation in vivo is known to be accompanied by acute secretion of ***insulin*** (47,48). The mechanism of (Beta)-AR-mediated **insulin** secretion is not fully understood. Recent studies have shown that a major part of this secretory response is mediated by (Beta)-ARs on adipocytes without direct stimulation of pancreatic (Beta)-cells (43). Because chronically increased **insulin** secretion has been proposed to contribute to the development of ***insulin*** resistance (23) and (aP2.sup.-/-) mice are protected from hyperinsulinemia and **insulin** resistance (14), we wondered whether alterations exist in the **insulin** secretory response to (Beta)-agonists in these animals. Indeed, we observed a profoundly blunted ***insulin*** response to CL 316,243 in (aP2.sup.-/-) mice investigated in the resting state. Whereas the basal ***insulin*** levels were similar in both groups, stimulated ***insulin*** levels of (aP2.sup.-/-) mice (7.5 (+ or -) 1.05 ng/ml) reached only 39% of the levels seen in (aP2.sup.+/+) controls (19.4...

...3 vs. 150 (+ or -) 7.3 mg/dl, (aP2.sup.+/+) vs. (aP2.sup.-/-); n = 13). An even stronger effect was observed after administration of isoproterenol. ***Insulin*** levels rose to 23.3 (+ or -) 2.4 ng/ml in (aP2.sup.+/+) mice but only to 5.7 (+ or -) 0.9 ng/ml in...

...0001) (Fig. 4). This diminished response in the (aP2.sup.-/-) mice was also reflected in systemic levels of C-peptide, a more accurate indicator

of ***insulin*** secretion than ***insulin*** itself. While basal levels were equal in both genotypes (352 (+ or -) 26 vs. 370 (+ or -) 18 pmol/l in (aP2.sup.-/-) vs. (aP2.sup.+/+) animals...

...0.005; n = 7) after administration of CL 316,243.

(Figure 4 ILLUSTRATION OMITTED)

One potential cause for the observed attenuation of (Beta)-AR-mediated ***insulin*** secretion in (aP2.sup.-/-) mice is a general reduction in pancreatic (Beta)-cell activity. To test this hypothesis, we performed **insulin** secretion experiments using alternative **insulin** secretagogues including arginine (1 g/kg) or a combination of arginine and the sulfonylurea glyburide (0.2 mg/kg). After intraperitoneal administration of arginine, systemic **insulin** concentrations were similar in (aP2.sup.-/-) and (aP2.sup.+/+) mice (4.7 (+ or -) 0.41 vs. 6.1 (+ or -) 0.94 ng/ml) (Fig. 4...

...levels in (aP2.sup.-/-) and (aP2.sup.+/+) mice (16.6 (+ or -) 1.2 vs. 16.1 (+ or -) 1.6 ng/ml) (Fig. 4).

Because altered **insulin** secretion might have additional effects on physiologic parameters in the postprandial period compared with the resting period, we also investigated (Beta)-AR-mediated **insulin** secretion in mice under this metabolic condition. As expected, basal ***insulin*** levels were elevated in postprandial animals (Table 1). On injection of CL 316,243, blood ***insulin*** levels rose in (aP2.sup.-/-) and (aP2.sup.+/+) mice (Table 1). However, significantly higher ***insulin*** concentrations were observed in (aP2.sup.+/+) compared with (aP2.sup.-/-) mice (8.4 (+ or -) 2.4 vs. 3.3 (+ or -) 0.9 ng/ml; P (is less than) 0.05).

Systemic concentrations of **insulin** secretagogues in response to (Beta)3-adrenergic stimulation. Experimental elevation of systemic FFAs has been shown to stimulate **insulin** secretion in vivo, making FFAs likely candidates for adipose-derived **insulin** secretagogues associated with (Beta)3-adrenergic stimulation. Total FFA levels appeared not to be responsible, however, for the difference in **insulin** secretion between the genotypes in our experiments where, in the resting state, systemic FFA levels were equal or even higher in (aP2.sup.-/-) vs. (aP2.sup.+/+) mice (Fig. 1B). Therefore, we tested whether the observed suppression of **insulin** secretion might be due to altered blood levels in (aP2.sup.-/-) mice of other known secretagogues. To determine a kinetic profile of **insulin** and these secretagogues, blood was collected from untreated animals and 5, 10, or 15 min after intraperitoneal administration of CL 316,243. As shown in Fig. 5A, ***insulin*** began to rise 5 min after (Beta)3-adrenergic stimulation. The average blood ***insulin*** concentration in the (aP2.sup.-/-) mice was already significantly lower at that point compared with the (aP2.sup.+/+) controls. ***Insulin*** continued to increase up to 15 min after injection, where the difference between the genotypes was most striking (Fig. 5A). Basal blood glucose levels were...

...Fig. 5B). After (Beta)3-adrenergic stimulation, no significant difference in blood glucose levels was evident between the two genotypes (Fig. 5B), despite strikingly different ***insulin*** concentrations, suggesting a difference in ***insulin*** sensitivity. Basal FFA concentrations were moderately but significantly higher in (aP2.sup.-/-) compared with (aP2.sup.+/+) mice (0.46 (+ or -) 0.03 vs. 0.32...

...CL 316,243 and increased only slightly thereafter, with no significant difference between the genotypes (Fig. 5C). Recent studies have shown that leptin can suppress ***insulin*** secretion (49-53). To test a potential role of leptin, we also measured serum leptin levels before and after the injection of CL 316,243...

...the experiment (Fig. 5D).

(Figure 5 ILLUSTRATION OMITTED)

Recently published data indicate that the endogenous subtypes of fatty acids differ in their potential to stimulate **insulin** secretion from pancreatic (Beta)-cells (54). Since absence of aP2 might change the composition, and therefore the insulinotropic potency, of FFAs released from adipocytes, we...

...Omega)9) (30% decrease in (aP2.sup.-/-); P (is less than) 0.05).

(Figure 6 ILLUSTRATION OMITTED)

aP2 expression in pancreatic (Beta)-cells. Because the ***insulin*** secretion pattern in (aP2.sup.-/-) mice is significantly altered, it is critical to determine whether the aP2 gene is expressed in pancreatic islets. Previous studies...

...HNF4(Alpha), a gene known to be expressed in these cells.

(Figure 7 ILLUSTRATION OMITTED)

DISCUSSION

In this study, we examined lipolysis and the associated **insulin** secretion in mice lacking aP2 and their wild-type counterparts. Experiments performed under various physiologic conditions consistently demonstrated an attenuated rise of glycerol in the...sup.-/-) adipocytes.

Stimulation of lipolysis with 6-AR-specific agonists such as CL 316,243 and isoproterenol is accompanied by an acute increase in blood ***insulin*** (47,48,62). Surprisingly, this ***insulin*** response was profoundly reduced in (aP2.sup.-/-) mice (Fig. 4; Table 1). A similar reduction of C-peptide levels in (aP2.sup.-/-) mice after (Beta)-adrenergic stimulation indicates that the defect is, at least partially, at the level of ***insulin*** secretion. This decreased responsiveness is unlikely to be through an indirect mechanism such as generally diminished activity of pancreatic (Beta)-cells (63-65), since arginine or a combination of arginine and glyburide stimulated **insulin** secretion to a similar extent in (aP2.sup.-/-) and (aP2.sup.+/+) mice (Fig. 4). Also, the reduced response is probably not related to glucose, which is a weak **insulin** secretagogue in the mouse strain used (C57B1/6) (66). We also tested whether a previously unrecognized expression of aP2 in pancreatic islets might be a...

...excluding the possibility of a direct impact of aP2 deficiency at that site.

Previous studies have suggested that the signal for (Beta)-AR stimulation of **insulin** secretion originates from adipocytes (43) and that it involves fatty acids (25,54,67,68). However, the strikingly different ***insulin*** response seen in the (aP2.sup.-/-) mice in our study occurred under experimental conditions in which significant differences in blood concentrations of total FFAs compared...

...controls were not evident (Fig. 5). This prompted us to examine specific fatty acids, since recently published experiments using perfused rat pancreas showed that the **insulin** secretory potential of individual fatty acid species is quite different, increasing with length and saturation of the hydrocarbon chain (54). In our experiments, we did...

...11-Eicoseneic acid has not been determined. Therefore, it is not clear whether these alterations are physiologically relevant and might account for the profoundly reduced ***insulin*** response in (aP2.sup.-/-) mice.

In this study, we observed decreased release of both glycerol and FFAs in response to dibutyryl cAMP stimulation in (aP2...

...Regardless, it is clear from the data shown here that under conditions where systemic FFA levels are similar, a dramatic difference is still detectable in **insulin** secretion in response to lipolytic stimuli in aP2-deficient animals. While it is possible to argue that the net local FFA input to the pancreatic possibilities emerge in view of the observed impact

of aP2 deficiency on ***insulin*** secretion. First, these results provide strong support for the notion of an endocrine axis between adipose tissue and the pancreas, demonstrating that a specific and isolated defect of adipose tissue can have a profound effect on the latter organ. Second, since hyperinsulinemia can lead to peripheral **insulin** resistance, (aP2.sup.-/-) animals might be protected from the development of obesity-induced **diabetes**, at least in part, through a reduction in ***insulin*** secretion triggered by adipocyte products. Currently, the mechanisms underlying increased peripheral **insulin** sensitivity in the absence of aP2 are not fully understood. Further characterization of these pathways might therefore provide important insights into the pathophysiology of type 2 **diabetes** and facilitate the development of novel therapeutic targets.

Note added in proof. During the review of this manuscript, Coe et al. (J Lipid Res 40...

...to membranes. Biochemistry 34:11840-11845, 1995

(14.) Hotamisligil GS, Johnson RS, Distel R J, Ellis R, Papaioannou VE, Spiegelman BM: Uncoupling of obesity from **insulin** resistance through a targeted mutation in aP2, the adipocyte fatty acid binding protein. Science 274:1377-1379, 1996

(15.) Krieg P, Feil S, Furstenberger G...

...in rat adipocytes. J Pharmacol Exp Ther 262:552-558, 1992

(19.) Londos C: Hormone-sensitive lipase and the control of lipolysis in adipocytes. In ***Diabetes*** Mellitus. A Fundamental and Clinical Text. LeRoith D, Taylor SI, Olefsky JM, Eds. Philadelphia, PA, Lippincott-Raven, 1996, p. 223-227

(20.) Stralfors P, Olsson droplets. J Biol Chem 266: 11341-11346, 1991

(23.) McGarry JD: What if Minkowski had been ageusic? An alternative angle on ***diabetes*** . Science 258:766-770, 1992

(24.) Ferrannini E, Barrett EJ, Bevilacqua S, DeFronzo RA: Effect of fatty acids on glucose production and utilization in man...

...72:1737-1747, 1983

(25.) Madison LL, Seyffert WA Jr, Unger RH, Barker B: Effect on plasma free fatty acids on plasma glucagon and serum **insulin** concentrations. Metabolism 17:301-304, 1968

(26.) Roden M, Price TB, Perseghin G, Petersen KF, Rothman DL, Cline GW, Shulman GI: Mechanism of free fatty acid-induced **insulin** resistance in humans. J Clin Invest 97:2859-2865, 1996

(27.) Boden G: Role of fatty acids in the pathogenesis of ***insulin*** resistance and NIDDM. ***Diabetes*** 46:3-10, 1997

(28.) Rodbell M: Metabolism of isolated fat cells. I. Effects on glucose metabolism and lipolysis. J Biol Chem 239:375-380...

...ribonuclease. Biochemistry 18:5294-5299, 1979

(33.) Hotmnisligil GS, Shargill NS, Spiegelman BM: Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked **insulin** resistance. Science 259:87-91, 1993

(34.) Laborda J: 36B4 cDNA used as an estradiol-independent mRNA control is the cDNA for human acidic ribosomal...

...4 (HNF-4). Biochim Biophys Acta 1131:211-213, 1992

(36.) Hotamisligil GS, Budavari A, Murray D, Spiegelman BM: Reduced tyrosine kinase activity of the **insulin** receptor in obesity-***diabetes*** . Central role of tumor necrosis factor-alpha. J Clin Invest 94:1543-1549, 1994

(37.) Dole VP, Meinertz H: Microdetermination of long-chain fatty acids...

...amino) propyl)-1,3-benzodioxole-2,2-dicarboxylate (CL 316,243): a potent beta-adrenergic agonist virtually specific for beta 3 receptors: a

promising anti- ***diabetic*** and antiobesity agent. J Meal Chem 35:3081-3084, 1992

(40.) Nahmias C, Blin N, Elalouf JM, Mattei MG, Strosberg AD, Emorine LJ: Molecular characterization...

...Hagen J, Cunningham BA, Corkey BE, Lowell BB: Beta3-adrenergic receptors on white and brown adipocytes mediate beta3-selective agonist-induced effects on energy expenditure, **insulin** secretion, and food intake: a study using transgenic and gene knockout mice. J Biol Chem 272:17686-17693, 1997

(44.) Fain JN, Kovacev VP, Scow RO: Antilipolytic effects of ***insulin*** in isolated fat cells of the rat. Endocrinology 78:773-778, 1966

(45.) Degerman E, Belfrage P, Manganiello VC: Structure, localization, and regulation of cGMP...

...neutral lipids. J Biol Chem 272:9378-9387, 1997

(47.) Yoshida T: The antidiabetic beta 3-adrenoceptor agonist BRL 26830A works by release of endogenous ***insulin*** . Am J Clin Nutr 55:237S-241S, 1992

(48.) Pinkney JH, Coppack SW, Mohamed-Ali V: Effect of isoprenaline on plasma leptin and lipolysis in humans. Clin Endocrinol 48: 407-411, 1998

(49.) Kieffer TJ, Heller RS, Leech CA, Holz GG, Habener JF: Leptin suppression of **insulin** secretion by the activation of ATP-sensitive K+ channels in pancreatic beta-cells. ***Diabetes*** 46:1087-1093, 1997

(50.) Kulkarni RN, Wang ZL, Wang RM, Hurley JD, Smith DM, Ghatei MA, Withers DJ, Gardiner JV, Bailey CJ, Bloom SR: Leptin rapidly suppresses **insulin** release from insulinoma cells, rat and human islets and, in vivo, in mice. J Clin Invest 100:2729-2736, 1997

(51.) Pallett AL, Morton NM, Cawthorne MA, Emilsson V: Leptin inhibits **insulin** secretion and reduces **insulin** mRNA levels in rat isolated pancreatic islets. Biochem Biophys Res Commun 238:267-270, 1997

(52.) Chen NG, Swick AG, Romsos DR: Leptin constrains acetylcholine-induced **insulin** secretion from pancreatic islets of ob/ob mice. J Clin Invest 100: 1174-1179, 1997

(53.) Emilsson V, Lin YL, Cawthorne MA, Morton NM, Davenport M: Expression of the functional leptin receptor mRNA in pancreatic islets and direct inhibitory action of leptin on ***insulin*** secretion. **Diabetes** 46:313-316, 1997

(54.) Stein DT, Stevenson BE, Chester MW, Basit M, Daniels MB, Turley SD, McGarry JD: The insulinotropic potency of fatty acids...10986, 1998

(56.) Kane CD, Coe NR, Vanlandingham B, Krieg P, Bernlohr DA: Expression, purification, and ligand-binding analysis of recombinant keratinocyte lipid-binding protein (**MAL**-1), an intracellular lipid-binding found overexpressed in neoplastic skin cells. Biochemistry 35:2894-2900, 1996

(57.) LiCata VJ, Bernlohr DA: Surface properties of adipocyte...

...J Biol Chem 270:29483-29492, 1995

(63.) Zhou YP, Grill VE: Long-term exposure of rat pancreatic islets to fatty acids inhibits glucose-induced **insulin** secretion and biosynthesis through a glucose fatty acid cycle. J Clin Invest 93:870-876, 1994

(64.) Unger RH: Lipotoxicity in the pathogenesis of obesity-dependent NIDDM: genetic and clinical implications. ***Diabetes*** 44:863-870, 1995

(65.) Brun T, Assimacopoulos-Jeannet F, Corkey BE, Prentki M: Long-chain fatty acids inhibit acetyl-CoA carboxylase gene expression in the pancreatic beta-cell line INS-1. ***Diabetes*** 46:393-400, 1997

(66.) Kaku K, Fiedorek FT Jr, Province M, Permutt MA: Genetic analysis of glucose tolerance in inbred mouse strains: evidence for polygenic control. ***Diabetes*** 37:707-713, 1988

(67.) Crespin SR, Greenough WBD, Steinberg D: Stimulation of

insulin secretion by long-chain free fatty acids: a direct pancreatic effect. J Clin Invest 52: 1979-1984, 1973

(68.) Goberna R, Tamarit J Jr, Osorio J, Fussganger R, Tamarit J, Pfeiffer EF: Action of B-hydroxy butyrate, acetoacetate and palmitate on the ***insulin*** release in the perfused isolated rat pancreas. Horm Metab Res 6:256-260, 1974

From the Division of Biological Sciences and Department of Nutrition (L...